

Science Skills Boot Camp

Workshop 2: Asking Scientific Questions

Keren Witkin, PhD

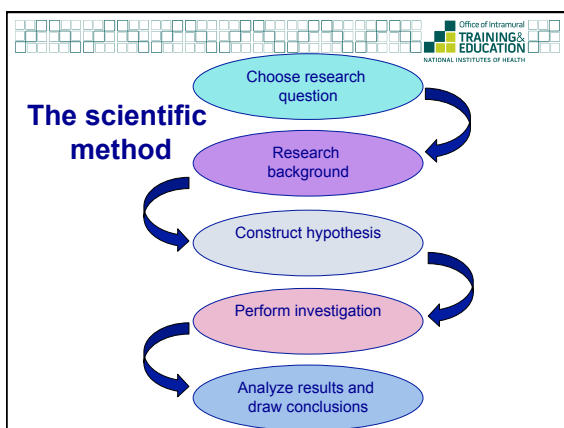
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“Science is a way of thinking
much more than it is a body of
knowledge.”

Carl Sagan



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Where does this fit into the
scientific method:

Prove that your
hypothesis is true

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Today's investigation:

Phenylthiocarbamide (PTC)

The human gene TAS2R38 encodes
taste receptor that detects PTC

<http://learn.genetics.utah.edu/content/begin/traits/ptc/>

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Class data collection 1:

Taste the PTC paper and pick one
description:

- ✧ This tastes like paper
- ✧ This tastes a little bitter
- ✧ This tastes horribly bitter

Please dispose of used PTC paper into the ziploc.

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Step 1:
Choosing a
research
question

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What makes a good research question?

- Testable
- Not too broad, but not too narrow
- Fits appropriately into the existing information
- Realistic in terms of resources
- Contributes to the field no matter what you find

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Some possible questions:

- How does the sense of taste work?
- Do factors other than genetics affect the ability to taste PTC?
- How did PTC sensitivity evolve?
- Are supertasters cuter than non-tasters?
- Are there gender differences in PTC sensitivity?
- Do PTC tasters have a specific DNA polymorphism?

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Step 2:
Researching
background

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Finding background information

- Primary literature
- Review articles
- Textbooks
- Your colleagues
- Internet resources

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Review articles are a great place to start!

- Provide an overview of the field
- Often written by experts in the field
- Summarize many primary papers
- Often contain useful diagrams

A Open mitosis

Centriosome Microtubule Chromosome

Webster et al. Journal of Cell Science. 2009. 122: 1477-1486

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Primary literature

- This is where the actual data is!
- Describes the experiments in detail
 - Introduction
 - Methods
 - Results
 - Figures
 - Tables
 - Discussion
 - References

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All about PubMed

- Free database of biomedical and life sciences literature
- Maintained by the National Library of Medicine
- Contains over 20 million citations
- <http://www.ncbi.nlm.nih.gov/pubmed/>

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phenylthiocarbamide

Display Settings Summary, 20 per page, Sorted by Recently Added

Filter your results: All (1054) Free Full Text (108) Review (43) Manual Filter

Results: 1 to 20 of 1054

1. Genetic variation in the hTAS2R38 taste receptor and brassica vegetable intake. Gorovic N, Atzai S, Tjenneland A, Overvad K, Vogel U, Albrechtsen C, Poulsen HE. *Scand J Clin Lab Invest*. 2011 Feb 21. [Epub ahead of print]. PMID: 21338274 [PubMed - as supplied by publisher] [Related citations](#)

2. Bitter receptor gene (TAS2R38) P49A genotypes and their associations with aversion to vegetables and sweetener foods in Malaysian subjects. Ooi SX, Lee PL, Law HY, Say YH, Aza Pae J. *Clin Nutr*. 2010;18(4):491-6. PMID: 21147709 [PubMed - indexed for MEDLINE] [Related citations](#)

3. Purification and characterization of phenoloxidase from the hemocytes of *Eurygaster integriceps* (Hemiptera: Scutelleridae). Ziaee A, Bandani AR, Malagoli D, Coma Brouhen Phylax B. *Biochem Mol Biol*. 2011 Jan;156(1):117-23. Epub 2010 Oct 20. PMID: 20970518 [PubMed - in process] [Related citations](#)

4. Sensitivity to bitter and sweet taste perception in schoolchildren and their relation to dental caries. Furukawa TR, Pahl-Friedrich RC, Maciel SM, Gontim-Junior A, Walter LR. *Oral Health Prev Dent*. 2010;8(3):253-9. PMID: 20849003 [PubMed - indexed for MEDLINE]

Also try: phenylthiocarbamide taste the genetics of phenylthiocarbamide perception phenylthiocarbamide gene

Titles with your search terms: The molecular basis of individual differences in phenylthiocarbamide and propyl isothiocyanate perception (J Neurosci. 2004) Positional cloning of the human quantitative trait locus underlying taste sensitivity (J Neurosci. 2003) Bitter taste study in a sardinian genetic isolate supports the association of (Chem Senses. 2004)

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3. Primary hemocyte culture of *Penaeus monodon* as an in vitro model for white spot syndrome virus (WSSV) and immune related gene expression and cytotoxicity assays. Jose S, Mohandas A, Philip R, Brijesh Singh B, J Invertebr Pathol. 2010 Nov;105(3):212-21. Epub 2010 Aug 31. PMID: 20907337 [PubMed - indexed for MEDLINE] [Related citations](#)

4. Identification of non-taster Japanese macaques for a specific bitter taste. Suzuki N, Sugawara T, Matsui A, Go Y, Hirai H, Imai H. *Primates*. 2010 Oct;51(4):285-9. Epub 2010 Jul 28.

Titles with your search terms: Bitter taste study in a sardinian genetic isolate supports the association of (Chem Senses. 2004) Associations between phenylthiocarbamide gene polymorphisms and phenolic food intake (J Nutr. 2005) See more...

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Varian in the gene TAS2R38 is associated with the eating behavior distribution in (Japost. 2010) Bitter taste perception in Neanderthals through the analyses of the TAS2R38 gene (Sci Lett. 2009) Prevalence of common disease-associated variants in Asian Indians (BMJ-Gene. 2008) See all (12)

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If there's no link from PubMed, the NIH library may still have it

- Go to the NCI Frederick Scientific Library webpage: <http://www-library.ncicrf.gov/default.aspx>
- Click on Online Journals (under Resources)



The screenshot shows the NCI Frederick Scientific Library homepage. The top navigation bar includes 'Home', 'About Us', 'Contact Us', 'Search', 'Help', 'Log Out', and 'My Account'. The main content area features a 'SPOTLIGHTS' section with several news items, including 'New Option for CDFR Access', '8th Annual NCI Publications Research Library Roundtable', and '2011 Updates & New Titles'. A sidebar on the left contains 'Quick Links' and 'New & Forthcoming' sections. The footer includes contact information for the library and logos for NCI, NIH, and the University of Maryland.

Where else can you find scientific papers?

- EMBASE: Biomedical and pharmacological database
- SCOPUS: Broad coverage of scientific, technical, medical, and social science literature, including arts and humanities
- Web of Science: Coverage of ~12,000 journals and conference proceedings. Not limited to biomedical sciences.

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More bioinformatics tools

- Sequence alignment programs
- Structure prediction programs
- Gene expression and regulation databases
- Organism-specific databases
- Pathway analysis
- Promoter/SNP prediction

And many others!

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Step 3: Constructing a hypothesis

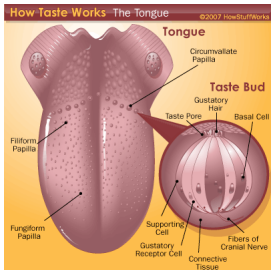
Hypothesis:
Tentative statement predicting the outcome of an experiment

If . . . , then . . .

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What we know about taste:

Taste is controlled by receptors in the tongue



The diagram illustrates the tongue's structure, including the circumvallate papilla, taste bud, gustatory hair, taste pore, basal cell, supporting cell, gustatory receptor cell, and fibers of the oropharyngeal nerve. It also shows the fungiform papilla and filiform papilla.

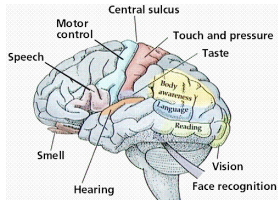
<http://health.howstuffworks.com>

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What we know about taste:

Taste perception involves additional factors

- Smell
- Temperature
- Visual cues
- Texture




The diagram shows the brain with various regions labeled: Central sulcus, Motor control, Speech, Smell, Hearing, Touch and pressure, Taste, Body awareness, Language, Reading, Vision, and Face recognition.

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What we know about taste:


Studies show that smelling **mint** while eating makes it difficult to identify food by taste.



Hypothesis:
If you smell mint while tasting PTC paper, **then** you will be less sensitive to the bitter taste.

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Step 4: Perform investigation



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graph TD
    A([Step 4:  
Perform investigation]) --> B([Planning])
    A --> C([Doing])
  
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Planning experiments

For each experiment:

- Define your objective
- Plan your general strategy
- Decide on experimental details

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Planning experiments

For each experiment:

- Define your objective
 - To determine whether strong smells decrease sensitivity to PTC paper
- Plan your general strategy
 - Participants will taste PTC paper and report on the taste. They will then smell mint while re-tasting PTC paper and report on the taste.
- Decide on experimental details
 - How many participants? What kind of mint? How will they rate PTC taste? What will be the controls?

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What are some features of a good experiment?

- Has a clear purpose
- Answers one question definitively
- Has appropriate **controls**
- Has limited **variables**
- Has a large enough sample size
- Uses available reagents and equipment
- Can be repeated by you and others

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Common controls

- **Positive controls**

Show that everything is working well, and that your conditions are able to achieve a positive result, even if your samples turn out all negative
- **Negative controls**

Show the base-line background in your experiment, using known samples that should produce a negative result

Sample

- + Sample

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Our experiment today:

Each subject will taste PTC paper and report whether there is no taste, weak bitter taste, or strong bitter taste

Experimental Group

Half of the subjects will re-taste PTC paper while smelling mint and will report whether there is no taste, weak bitter taste or strong bitter taste

Control Group

Half of the subjects will re-taste PTC paper while smelling parsley and will report whether there is no taste, weak bitter taste or strong bitter taste

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- What are the controls in this experiment?
- What are the variables?
- Are there other controls that we're missing?



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Tips for good experiments

- Be prepared
- Be organized
- Be meticulous
 - Work deliberately and carefully
 - Follow protocol closely
 - Note any deviations from protocol
- Minimize bias
- Document everything

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Learning a new technique

- Find a protocol
- Read it carefully
- Consult with your mentor or other experts
- Make or acquire reagents ahead of time
- Learn how to use required equipment
- Do a "dry run"
- Allow plenty of time for the first run

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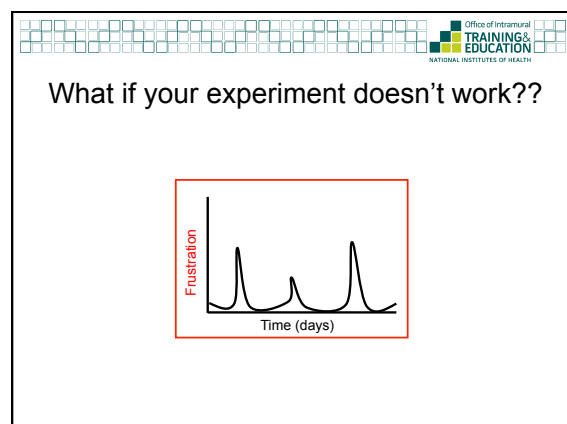
Making or acquiring reagents

- If ordering reagents, do it as early as possible
- Research how each reagent should be used and stored
- If making up solutions
 - Make sure you know what solvent to use
 - Brush up on molarity and serial dilutions, if necessary
 - Check and re-check all calculations

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Common mistakes

- Doing huge experiments with too many samples
- Not thinking carefully about your controls before you start
- Waiting until the last minute before tracking down reagents
- Forgetting to grow up the cells you need ahead of time



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When your experiment doesn't work: Troubleshooting

- Identify possible sources of error
 - Reread protocol
 - Check calculations
 - Consider whether reagents or equipment might be suspect
 - Think about repeating experiment as is
- Consult with mentor
- Consider whether your hypothesis might be flawed
- Don't get frustrated!

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Class data collection- Part 2

1. Taste the PTC paper while smelling the mint or parsley and pick one description:
 - ✧ This tastes like paper
 - ✧ This tastes a little bitter
 - ✧ This tastes horribly bitter
2. Dispose of all waste into the ziploc
3. Class data will be collected and recorded on the board

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Step 5:
Analyzing
Results and
Drawing
Conclusions

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- How many people changed their responses after smelling the mint?
- How many people changed their responses in the control group?
- Can we draw any conclusions?
- Does our data suggest future experiments?
- How do we want to present this data?

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Raw data: Recorded in your notebook

Subject	Mint or parsley	Before	After
1	M	No	No
2	M	Strong	Strong
3	M	Strong	Weak
4	M	Weak	Weak
5	P	No	No
6	P	Weak	Strong
7	P	Weak	Weak

Other types of raw data:
Gels
Blots
Photographs
Observations

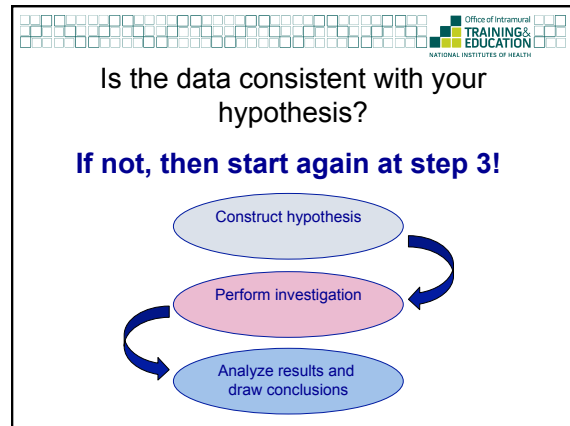
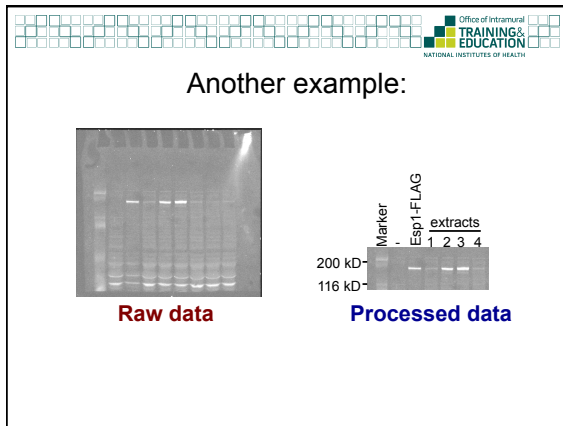
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Processed data:

	Parsley		Mint	
	Before	After	Before	After
No taste	12	12	13	13
Weakly bitter	27	25	23	24
Strongly bitter	11	13	14	13

PTC tasting results

Category	Before	After
no taste (control)	12	12
no taste (mint)	13	13
weakly bitter (control)	27	25
weakly bitter (mint)	23	24
strongly bitter (control)	11	13
strongly bitter (mint)	14	13



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Data vs interpretation

Data: 48/50 study participants reported no change in PTC taste sensitivity after smelling mint, compared to 49/50 in the control group.

Interpretations:

- Smell does not affect taste
- Smelling mint does not affect taste
- The smell of mint does not affect PTC taste sensitivity

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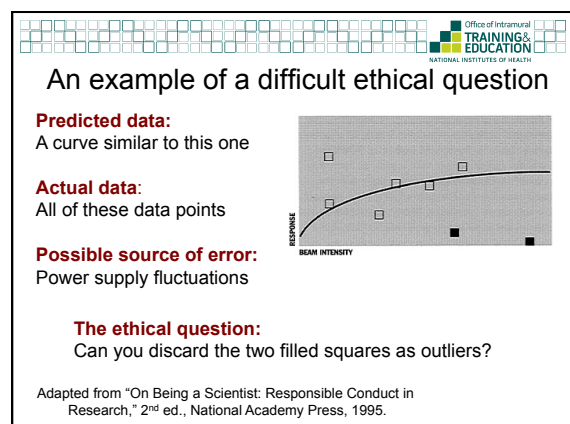
Question:

How could we design an experiment to better test the broader conclusion that **smell does not affect taste?**

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Does your data mean what you think it means?

- Is it statistically significant?
- Are you doing the right statistical analysis?
- Do you have a large enough sample size or enough repetitions?
- Are there alternative explanations?
- Are there confounding factors?



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Pitfalls in data analysis

- Only considering specific data points
- Over-interpretation of data
- Ignoring confounding factors
- Using too small of a sample size

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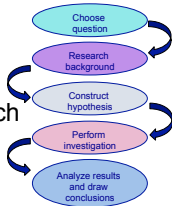
Scientific misconduct

- **Falsifying data**
Can happen accidentally when you “process” data
- **Fabricating data**
This is always wrong!
- **Plagiarism**
Includes using other’s IDEAS as well as WORDS

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Same basic scientific method no matter what kind of research you do

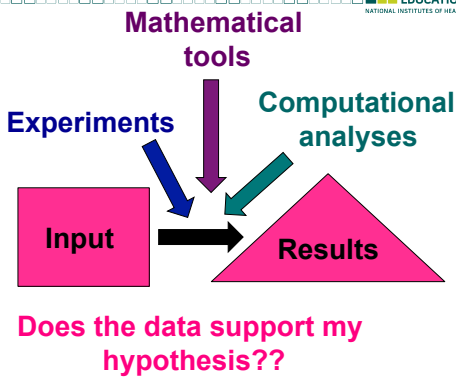
- Basic research
- Clinical research
- Translational research
- Social and behavioral research
- Epidemiology
- Computational research
- Mathematical modeling



```

graph TD
    A(Choose question) --> B(Research background)
    B --> C(Construct hypothesis)
    C --> D(Perform investigation)
    D --> E(Analyze results and draw conclusions)
    E --> A
  
```

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Does the data support my hypothesis??

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Special considerations for epidemiologists

- Are you surveying an appropriate population?
- Do you have enough study participants?
- Are you using the appropriate analytical tools?
- Have you considered potential alternative explanations?
 - Confounding factors
 - Bias
 - Chance
 - Reverse causality

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Special considerations for clinical research

- Bioethics
- Professionalism
- Confidentiality
- Possibility of health risks for investigator
- Critical to have appropriate study design and meticulous technique
 - Institutional Review Board (IRB)
 - Data Safety Monitoring Board
 - Double-blind studies

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More on **bioethics**: The 7 major ethical principles that guide clinical research

Each study must have:

- Social and clinical value
- Scientific validity
- Fair subject selection
- Favorable risk-benefit ratio
- Independent review
- Informed consent
- Respect for potential and enrolled subjects

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Special considerations when using animals in research

- Oversight by the NIH Office of Laboratory Animal Welfare
- Must have an Animal Care and Use Protocol
- Ensuring humane and responsible use
 - Carefully designed experiments
 - Minimizing the number of animals used
 - Avoiding/minimizing pain and stress
 - Appropriate housing conditions
 - Appropriate sedation or anesthesia
 - Veterinary care, when necessary

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Ethical considerations for all kinds of scientists

- Honesty
- Objectivity
- Integrity
- Carefulness
- Respect for intellectual property
- Responsible publication
- Respect for colleagues

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Last but not least . . .

Communicate your results